Liver Pathology Resulting from Therapeutic Dose of Terbinafine in Rats

San-Duo Chen¹ Chi-Chung Chou² Ming-Chih Chou³ William Bell⁴ C. Howard Tseng⁵ Jen-Hung Yang⁶*

Terbinafine has been suggested as the treatment of choice for dermatophyte onychomycosis, and has excellent tolerability in treated patients. Hepatic injury has been reported with the use of terbinafine, however, the mechanism of hepatotoxicity is still obscure. The present study was designed to investigate the pathology of the liver in rats following administration of the therapeutic dose of terbinafine 4 mg/kg b.w. p.o., daily. The hemogram and biochemistry including liver enzymes were all normal in all groups of rats following treatment, except for a transient increase in the level of alkaline phosphatase in rats treated with terbinafine after 2 weeks. The histology of the liver revealed fatty change in terbinafine-treated rats at 6 weeks. Ultrastructurally, only a few lipid droplets in occasional hepatocytes were found at 2 weeks. However, abundant lipid droplets with deposition of amorphous electron-dense substances were found in hepatocytes in rats treated for 6 weeks. Bile was found in the hepatocytes and in bile duct canaliculi. Moreover, the unique finding of a phagosome-like structure containing needle-shape substance was observed in hepatocytes. In conclusion, we demonstrated that terbinafine in therapeutic doses may have hepatotoxicity in rats. In comparison with liver enzyme tests and histological examination, electron microscopy is a more sensitive tool to detect the liver pathology.(Dermatol Sinica 22 : 274-280, 2004)

Key words: Terbinafine, Liver pathology, Deposits in hepatocytes
Terbinafine 4 mg/kg/day的臨床治療剂量，以探討肝臟的病理變化。在給與terbinafine二至六週的大白鼠，其血液與生化檢查均屬正常(除了alkaline phosphotase在二週時暫時升高外)，組織病理在六週時方顯現脂肪變病；電子顯微鏡檢查，二週時的肝細胞只有些許的脂肪滴，但是六週時的肝細胞則富含脂肪滴，並有高電子密度不定形物存在於肝細胞內。肝細胞與微小膽管也有膽汁滯留，此外，較為特殊的是發現肝細胞內出現含有針狀物質類似phagosome的胞器。本動物實驗顯示臨床治療劑量使用的terbinafine，對大白鼠可能有肝臟的毒性，相較於生化與組織學檢查，電子顯微鏡用以偵測肝臟病變是更為敏感的工具。(中華皮誌22：274-280, 2004)

INTRODUCTION

Terbinafine, an allylamine, inhibits the enzyme squalene epoxidase thus blocking the conversion of squalene to squalene epoxide in the biosynthetic pathway of ergosterol, an integral component of the fungal cell membrane.1-3 Terbinafine has demonstrated excellent fungicidal activity against the dermatophytes and has been suggested as the first-line treatment for onychomycosis.1, 3 Terbinafine is licensed at a dose of 250 mg daily for 6 weeks and 12 weeks in fingernail and toenail infection, respectively.1, 3

Although generally well tolerated, oral terbinafine is associated with adverse events in 10.5% of patients, primarily causing gastrointestinal or dermatological disorders.4 Hepatotoxicity may occur in patients with and without known pre-existing liver disease.3, 5 Rare cases with fatal hepatotoxicity or fulminant hepatic failure, some leading to death or requiring liver transplantation, have occurred with the use of terbinafine.5

It has been hypothesized that the terbinafine-associated liver toxicity is attributed to idiosyncracy, either through hypersensitivity or metabolic abnormality.6-12 However, the exact mechanism of liver toxicity is still obscure. The present study was designed to investigate the effects on the liver in rats following daily administration of therapeutic doses of terbinafine.

MATERIALS AND METHODS

ANIMALS AND MEDICATION

Twenty-four male Sprague-Dawley rats, 6-8 weeks old, weighing 250 to 300 g, were purchased from the Laboratory Animal Center, National Taiwan University. The 24 animals were equally divided into four groups A, B, C and D. The animals were housed in an air-conditioned room controlled at 25 ± 1 °C and 55 ± 10% relative humidity with a 12 hour-light/12-hour dark cycle, and were fed with pellet food (Pellet food feed for Rat, Fu-Shiou Food Inc, Taiwan) and water ad libitum.

Terbinafine (Lamisil®, Novartis Pharma AG, Basle, Switzerland) was purchased from the Jen-Ai Hospital Pharmacy for study. Petraný et al.13 found that an oral dose of 4-6 mg/kg b.w. daily would have excellent activity against trichophytosis. Thus, we employed the regimen of a daily dose of terbinafine 4 mg/kg b.w. which was also equivalent to the recommended human clinical dose (250 mg, qd) to proceed the experiments. The rats in experimental groups A and C, were fed orally with terbinafine via intragastric intubation for 2 and 6 weeks, respectively. The control groups B and D were fed via intragastric intubation with distilled water orally. The animals of group A and B were sacrificed at 2 weeks, and the animals of group C and D were sacrificed at 6 weeks.

HEMATOLOGICAL ANALYSIS

Blood samples were analyzed with Automatic Hematometer (Serono System 9000,
Hematometer, Japan) for hemogram that included WBC, RBC, Hb, Hct value, MCV, MCH, MCHC and platelet count. Blood chemistry was measured with Automatic Serobiochemical analyzer (Ciba-Corning Express Plus, U.S.A.), for asparate transaminase (AST), alanine transaminase (ALT), lactic dehydrogenase (LDH), alkaline phosphatase (Alk-P), gamma glutamyltranspeptidase (γGT), blood urea nitrogen (BUN) and creatinine. The hemogram and blood chemistry data were analyzed by the Duncan’s new multiple range test for statistical significance if \( p < 0.05 \).

**HISTOLOGY AND LIPID STAINING**

The tissue from the liver was fixed with formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin (H & E) for light microscopy.

The liver tissues were frozen in liquid nitrogen and sectioned with Cryostat, and stained with Sudan III and Sudan black for light microscopy.

**ELECTRON MICROSCOPY**

The liver tissues were fixed in 4% glutaraldehyde with 0.1 M phosphate buffer, pH 7.4, for 1 hour, and post-fixed in 1% OsO4 for 1

### Table I. Summary of the laboratory and pathological findings in the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats (N)</th>
<th>Hemogram</th>
<th>Biochemistry</th>
<th>Histology (incidence of abnormal findings)</th>
<th>Ultrastructure (incidence of abnormal findings)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>terbinafine-treated 2 weeks (6)</td>
<td>WNL (except Alk-P)</td>
<td>WNL</td>
<td>Negative (0/6)</td>
<td>A few lipid droplets in occasional hepatocytes. (6/6)</td>
</tr>
<tr>
<td>B</td>
<td>control 2 weeks (6)</td>
<td>WNL</td>
<td>WNL</td>
<td>Negative (0/6)</td>
<td>Negative (0/6)</td>
</tr>
<tr>
<td>C</td>
<td>terbinafine-treated 6 weeks (6)</td>
<td>WNL</td>
<td>WNL</td>
<td>fatty degeneration of liver (4/6)</td>
<td>Lipid droplets with deposits of electron-dense amorphous substances, and phagosome-like structures containing needle-shape substances in hepatocytes. Bile in hepatocyte and canaliculi. (6/6)</td>
</tr>
<tr>
<td>D</td>
<td>control 6 weeks (6)</td>
<td>WNL</td>
<td>WNL</td>
<td>Negative (0/6)</td>
<td>Negative (0/6)</td>
</tr>
</tbody>
</table>

\( N \) : Number of rats; WNL: within normal limits; WNL (except Alk-P): see Alk-P* in Table II.

### Table II. Biochemical data in the experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Rats (N)</th>
<th>AST (u/l)</th>
<th>ALT (u/l)</th>
<th>LDH (u/l)</th>
<th>Alk-P* (u/l)</th>
<th>γGT (u/l)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>terbinafine-treated 2 weeks (6)</td>
<td>148.8 ± 44.25</td>
<td>32.8 ± 4.20</td>
<td>1586 ± 727.5</td>
<td>407.7 ± 73.4</td>
<td>150 ± 0.84</td>
<td>10.52 ± 1.59</td>
<td>0.48 ± 0.08</td>
</tr>
<tr>
<td>B</td>
<td>control 2 weeks (6)</td>
<td>165.8 ± 33.9</td>
<td>35.8 ± 8.4</td>
<td>1768 ± 855</td>
<td>297.0 ± 110.0</td>
<td>2.24 ± 1.88</td>
<td>12.1 ± 2.01</td>
<td>0.54 ± 0.21</td>
</tr>
<tr>
<td>C</td>
<td>terbinafine-treated 6 weeks (6)</td>
<td>189.0 ± 33.9</td>
<td>41.6 ± 16.8</td>
<td>2600 ± 929</td>
<td>170.0 ± 56.0</td>
<td>3.25 ± 3.94</td>
<td>11.9 ± 1.65</td>
<td>0.66 ± 0.11</td>
</tr>
<tr>
<td>D</td>
<td>control 6 weeks (6)</td>
<td>156.0 ± 76.2</td>
<td>42.9 ±12.4</td>
<td>1965 ± 1326</td>
<td>255.2 ± 78.4</td>
<td>1.29 ± 1.11</td>
<td>13.7 ± 3.89</td>
<td>0.55 ± 0.27</td>
</tr>
</tbody>
</table>

\( N \) : Number of rats; AST: asparate transaminase; ALT: alanine transaminase; LDH: lactic dehydrogenase; Alk-P: alkaline phosphatase; γGT: gamma glutamyltranspeptidase; BUN: blood urea nitrogen.

Alk-P*: At 2 weeks, the value of the Alk-P \( \{ 407.7 ± 73.4 \text{ u/l} \} \) in terbinafine-treated animals was significantly higher than that \( \{ 297.0 ± 110.0 \text{ u/l} \} \) of the controls \( (p<0.05) \). However, at 6 weeks, the Alk-P value had no significant difference as compared to the control group.
hour. Tissues were then, dehydrated in graded alcohol, embedded in Spur's medium (SPI Supplies, Probe Inc., Box 342, West-Chester, PA19380, SPI# 2680 kit, U.S.A.), sectioned into 75 nm slices, stained with uranyl acetate and lead citrate, and examined in a Zeiss 900 transmission electron microscope (Carl Zeiss, Postfach 1369-80, D-7082, Berkochen, Germany).

RESULTS

HEMATOLOGICAL EXAMINATION

In comparison with the controls, the hemogram and the biochemical tests in terbinafine-treated rats (for 2 and 6 weeks, respectively) did not show statistical difference ($p > 0.05$), with the exception of alkaline phos- phatase at 2 weeks (Table I and Table II).

HISTOLOGY

No histopathological changes could be identified in the liver of the animals at 2 weeks, neither in terbinafine-treated nor the control group. The histology revealed fatty degeneration, confirmed by lipid stain (Fig. 1 & Fig. 2), in liver among two thirds (4 / 6) of the terbinafine-treated rats at 6 weeks (Table I).
ELECTRON MICROSCOPY

Ultrastructurally, only a few scattered lipid droplets could be demonstrated in the cytoplasm of hepatocytes of the terbinafine-treated rats at 2 weeks. Abundant lipid droplets with deposition of electron-dense amorphous substances were found in the hepatocytes of the terbinafine-treated rats at 6 weeks (Fig. 3). Vesiculation of endoplasmic reticulum was present in hepatocytes, and bile was seen in the cytoplasm of hepatocytes and in the bile duct canaliculi without inflammatory cells infiltrate. Of particular interest were the findings of the electron-dense amorphous substances in lipid droplets and needle-shape substances in phagosome-like structures in hepatocytes (Fig. 4 & 5) (Table I).

DISCUSSION

Terbinafine is extensively metabolized in the liver, and only a small fraction ( < 5%) of total cytochrome P450 capacity is involved in terbinafine metabolism, resulting in 15 metabolites, which are excreted in the urine and feces. The occurrence of fatal hepatotoxicity with terbinafine treatment is a rare event (1 in 120,000), but the occurrence of asymptomatic elevations of liver enzymes is relatively common (1 in 200 to 2.7% ) in patients treated with oral terbinafine. Little is known about the histopathological and ultrastructural changes of the liver in terbinafine-treated patients with asymptomatic elevations of liver enzymes. Furthermore, the mechanism of hepatotoxicity with terbinafine is unknown, and it is usually ascribed to idiosyncrasy, either by hypersensitivity or potential hepatotoxicity.

In this study, we demonstrate in the first time the hepatotoxic effects in animals treated with daily administration of therapeutic dose of terbinafine. At 2 weeks, we couldn't detect any abnormality either in hemogram, liver enzyme panel (except Alk-P) nor in hepatic histology. Employing electron microscopy, we could only demonstrate deposition of lipid droplets in occasional hepatocytes. At 6 weeks, the hemogram and liver enzyme panel were all normal when compared with the control animals. However, hepatic histology revealed prominent fatty degeneration in two thirds of the terbinafine-treated rats. Ultrastructurally, the liver injury became prominent as described above. In comparison to liver enzyme tests and histological examination, the electron microscopy is a more sensitive tool to detect liver pathology. In this study, the incidence of the hepatobiliary dysfunction among rats with terbinafine was fre-
quent. We thus suggested that terbinafine may have hepatotoxicity to animals receiving therapeutic doses for 6 weeks.

Terbinafine inhibits the enzyme squalene epoxidase thus blocking the synthesis of ergosterol, an essential constituent of fungal cell membrane, as is cholesterol in mammalian membranes. The rat liver squalene epoxidase was much less sensitive to terbinafine than the fungal enzyme, by a factor of three orders of magnitude. The selective and specific action of terbinafine on fungal squalene epoxidase suggests that mechanism-based adverse effects in mammals are unlikely. Recently, Iverson identified a reactive metabolite of terbinafine (TBF-A) conjugated with glutathione, which could play a role leading to direct liver toxicity or immune-mediated hepatobiliary dysfunction. We thus speculate that the electron-dense or needle-shaped contents in fat droplets may be due to accumulation of terbinafine metabolites, which subsequently result in hepatic injury. Further investigations to clarify the nature and the constituents of the lipid droplets and their electron-dense or needle-shaped contents may help to unravel the mechanism of the hepatotoxicity induced by terbinafine.

In conclusion, we demonstrated that therapeutic dosage of terbinafine may have hepatotoxicity in rats. In comparison with liver enzyme tests and histological examination, the electron microscopy is a more sensitive tool to detect the liver pathology. Further investigation is needed to elucidate in detail the mechanism of hepatotoxicity of terbinafine.

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